

## Office of Research Ethics & Integrity Institutional Animal Care and Use Committee (IACUC)

Policy: Use of Zebrafish Responsible University Office:

Office of Research Ethics & Integrity, Research Planning & Development

**Effective:** November 1, 2017 **Responsible University Administrator:** 

Last Updated: October 31, 2017 Vice President for Research & Graduate Studies

Date Approved: October 31, 2017 Policy Contacts: QU-IACUC

**Purpose:** The following policy has been developed to ensure compliance with the Animal Welfare Policies regulated by Ministry of Public Health and international regulations, and to ensure humane use and care of vertebrate animals. For the purposes of this policy, zebrafish are defined as members of the genus *Danio* and related species.

## Stages and definitions:

472 hours post fertilization - embryos
 3-29 days post fertilization - larvae
 30-89 days post fertilization - juveniles
 >90 days post fertilization - adults

**Policy:** The QU-IACUC must evaluate all experiments on zebrafish that are allowed to develop greater than 72 hours following fertilization. Any experiments performed on zebrafish larvae that are older than 72 hours must be described in an IACUC approved animal use protocol and the number used reported in the protocol. Furthermore, any experiments on embryos at less than 72 hours from fertilization must be described in an IACUC-approved protocol provided that those animals are expected to survive to greater than 72 hours in age.

Zebrafish embryos that are manipulated before the 72-hour limit and are euthanized before that time are not regulated by the IACUC; however, <u>in this case</u>, a Notice of Intent to Use Zebrafish Embryos protocol has to be filled and submitted to QU-IACUC for their records and tracking purposes.

Zebrafish of all ages, including embryos, must be euthanized in accordance with the AVMA<sup>1</sup> Guidelines for the Euthanasia of Animals.

**Rationale:** The development of the zebrafish embryo proceeds similarly to avian and mammalian species but the environment in which this happens is completely different. The zebrafish embryo develops externally to the mother and there is no calcified shell to protect the embryo because this would hinder fertilization. Female zebrafish spawn unfertilized eggs which are rapidly fertilized. The

<sup>&</sup>lt;sup>1</sup> https://www.avma.org/KB/Policies/Documents/euthanasia.pdf



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eggs sink to the bottom of the water. Although a one-cell embryo can survive outside the chorionic membrane if carefully removed, this is not the same as chick survival outside of the eggshell. Zebrafish embryos survive on nutrients from the yolk sack from days one through five. Although this may vary, depending on such factors as temperature, the IACUC decided that, in a laboratory situation, temperature variability would be minimized and would approach ideal conditions that allow the embryo to develop to a free feeding state by 72 hours. Additionally, the only practical way to apply policy was to select a period of time after fertilization from which larval numbers could be realistically counted, or at least estimated. Based on these considerations, the IACUC established a time point of 72 hours for viability of zebrafish larvae.

## Resources

- PHS Policy on Humane Care and Use of Laboratory Animals, FAQ's. Office of Laboratory Animal Welfare's (OLAW) FAQs, Section A, question 4 and question 5. <a href="https://grants.nih.gov/grants/olaw/faqs.htm">https://grants.nih.gov/grants/olaw/faqs.htm</a>
- 2. AVMA Guidelines for the Euthanasia of Animals: 2013 Edition. https://www.avma.org/KB/Policies/Documents/euthanasia.pdf
- 3. OLAW Online Seminar: Zebrafish 101 for IACUCs, March 12, 2015. https://grants.nih.gov/sites/default/files/150312\_Zebrafish\_slides.pdf
- Guidelines for the Use of Zebrafish in the NIH Intramural Research Program, <a href="https://oacu.oir.nih.gov/sites/default/files/uploads/arac-quidelines/zebrafish.pdf">https://oacu.oir.nih.gov/sites/default/files/uploads/arac-quidelines/zebrafish.pdf</a>
- 5. Bert et al. "Considerations for a European Animal Welfare Standard to Evaluate Adverse Phenotypes in Teleost Fish." *The EMBO Journal* 35, no. 11 (June 1, 2016): 1151–54. doi:10.15252/embj.201694448.
- Strähle et al. "Zebrafish Embryos as an Alternative to Animal experiments—A Commentary on the Definition of the Onset of Protected Life Stages in Animal Welfare Regulations." Reproductive Toxicology, Zebrafish Teratogenesis, 33, no. 2 (April 2012): 128–32. doi:10.1016/j.reprotox.2011.06.121.